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REVIEW

Impact of *NR5A2* and *RYR2* 3′UTR polymorphisms on the risk of breast cancer in a Chinese Han population

Ying Wei[1,2] · Xiaolin Wang[3] · Zhe Zhang[3] · Changtao Zhao[2] · Yuwei Chang[2] · Zhiqing Bian[2] · Xinhan Zhao[1]

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Abstract

Objectives The *NR5A2* and *RYR2* genes are important players in steroid metabolism and play an important role in cancer research. In this research, we want to evaluate the effect of *NR5A2* and *RYR2* polymorphisms on breast cancer (BC).

Methods Four single nucleotide polymorphisms on *NR5A2* and *RYR2* were selected to genotype by Agena MassARRAY in 379 BC patients and 407 healthy controls. Using the PLINK software to calculate the Odds ratio (OR) and 95% confidence intervals (CIs) via the logistic regression analysis to evaluate the risk for BC.

Results We found that *NR5A2* rs2246209 significantly decreased the risk of BC with the AA genotype (OR 0.58, 95%CI 0.34–0.99, $p=0.049$), and recessive model (OR 0.59, 95%CI 0.35–0.99, $p=0.046$); rs12594 in the *RYR2* gene significantly decreased the risk of BC in the GG genotype (OR 0.44, 95%CI 0.22–0.88, $p=0.020$), and recessive model (OR 0.43, 95%CI 0.21–0.85, $p=0.016$). Further stratification analysis showed that *NR5A2* rs2246209 was related to a lower incidence of BC affected by age, lymph nodes metastasis, and tumor stage; *RYR2* rs12594 was related to a decreased BC risk restricted by age, estrogen receptor (ER), progesterone receptor (PR), menopausal status, tumor size, and tumor stage. Rs12594 in the *RyR2* gene remained significant on the genetic susceptibility of PR-positive BC after Bonferroni correction ($p<0.0125$).

Conclusions This study provides an evidence *that NR5A2* rs2246209 and *RYR2* rs12594 decreased the risk of breast cancer.

Keywords Breast cancer · *NR5A2* gene · *RYR2* gene · Polymorphism · Chinese han population

Abbreviations

BC	Breast cancer
ER	Estrogen receptor
PR	Progesterone receptor
LNM	Lymph node metastasis
ORs	Odds ratios
CIs	Confidence intervals
SNP	Single nucleotide polymorphism
HWE	Hardy–Weinberg equilibrium

Introduction

Breast cancer (BC), a malignant tumor that occurs in breast epithelial tissue, is a major public health problem worldwide and one of the most common malignant tumors diagnosed in women [1], seriously affecting women's physical and mental health and even life threatening. In 2017, the global incidence of breast cancer increased to 1,960,681 cases [2]. According to the International Agency for Research on Cancer (IARC, https://www.iarc.fr/), there are 2.1 million new cases worldwide only in 2018, and the death toll exceeds 626 679, and 99% of which occur in women and only 1% in men [3, 4]. We all know that history of breast disease, family history of cancer, frequent abortions, taking contraceptives, and passive smoking are risk factors for breast cancer. Studies have found high inter-individual differences in BC susceptibility, clinical outcomes, and treatment response, highlighting the importance of genetic alterations in BC [5]. Previous studies have shown that some genes are related to breast cancer, such as Liver receptor homolog-1 (*LRH-1*) [6] and Growth Regulation by Estrogen in Breast Cancer 1 (*GREB1*) [7].

Ying Wei and Xiaolin Wang are Joint first authors.

✉ Xinhan Zhao
zhaoxinhan@mail.xjtu.edu.cn

[1] Department of Internal Medicine Oncology, The First Affiliated Hospital of Xi'an Jiaotong University School of Medicine, Xi'an 710061, Shaanxi, China

[2] Department of Internal Medicine Oncology, Yulin No.2 Hospital, Yulin 719000, Shaanxi, China

[3] Department of General Surgery, Yulin No.2 Hospital, Yulin 719000, Shaanxi, China

NR5A2 (Nuclear Receptor Subfamily 5 Group A Member 2), alias *LRH-1*, belongs to the nuclear receptor NR5A subfamily and is expressed in the development of endothelial origin and adult tissues [8], and acts as a transcription factor in embryogenesis, steroid and cholesterol metabolism, inflammation, and various cancers [9, 10]. A study by Ueno et al. in Japanese patients with pancreatic cancer found that SNPs in the *NR5A2* gene were associated with a reduced risk of pancreatic cancer [11]. Zhang et al. [12] found that the polymorphism of *NR5A2* gene was significantly associated with regional lymph node metastasis or distant metastasis in patients with gastric cancer. Xiao et al. [13] found that the expression of *NR5A2* gene was increased in clinical samples of hepatocellular carcinoma by immunohistochemistry, and that *NR5A2* gene was predicted to be a therapeutic target for hepatocellular carcinoma. Li-Yun Chang et al. showed that in the ER (−) and ER (−))/ER (+) mixed group, *NR5A2* expression plays an important role in the prognosis of breast cancer [14]. Jiang Zhu et al. verified that miR-27b-3p enhances the role of tamoxifen in breast cancer induction by inhibiting the expression of *NR5A2* and cAMP response element binding protein 1 (*CREB1*). The impact of *NR5A2* polymorphism on breast cancer risk in Chinese Han population has not been reported. Therefore, we determined that the *NR5A2* gene is worth exploring in BC.

The ryanodine receptor is a Ca-releasing channel protein in Ca cells that are sensitive to caffeine and ryandin. Previous studies have shown that the SR is the primary intracellular Ca^{2+} store and ryanodine receptors (*RyR*) are the Ca^{2+} release channel*s* [15]. Y Ogawa found that three subtypes of *RyR* have been identified in mammals: *RyR1*, *RyR2*, and *RyR3* [16]. *RYR2* (Ryanodine Receptor 2) is also an important subject in cancer research, and mutations of *RYR2* were associated with several cancers. Femi et al. [17] found that mutations in the *RYR2* gene affect the prognosis of cervical cancer, and speculate that *RYR2* can be used as a target for the treatment of cervical cancer. Cai et al. [18] found that *RYR2* gene mutation may affect lung adenocarcinoma immunodiagnosis and immunotherapy. Mansoor Abdul et al. indicate that *RYR* can be used as a prognostic indicator and / or target for breast cancer [19]. Lina Zhang et al. found that receptor gene 3 (*RYR3*) is associated with breast cancer risk and calcification [20].

The above findings support the associations between *NR5A2/RYR2* genetic polymorphisms and the risk of cancers. However, there were few obvious results in the relationship of *NR5A2/RYR2* gene with BC. Therefore, the aim of this study was to validate the genetic association of *NR5A2/RYR2* polymorphisms with the risk of BC in Chinese Han population.

Materials and methods

Study participants

Using a case–control design, 379 patients (mean age: 51.50 ± 10.14 years) with BC and 407 controls (mean age: 53.04 ± 9.29 years) were enrolled. All patients were recruited from Shaanxi Provincial Cancer Hospital. Patient inclusion criteria: BC was confirmed by physical examination, imaging (X-ray, color Doppler ultrasound, MRI), and histopathology and cytopathology. Patients with complex blood diseases, autoimmune diseases, trauma, or other tumors were excluded from this study. After that, we investigated and collected clinical indicators of the BC patients, including age, tumor size, tumor stage, and the statuses of estrogen receptor (ER), progesterone receptor (PR), Cerb-B2, lymph node metastasis (LNM), and menopausal.

The controls were healthy volunteers from Shaanxi Provincial Cancer Hospital (Xi´an, Shaanxi, China) recruited during the same period. Inclusion criteria of control group included no medical or family history of cancer or any neurogenic diseases or any breast abnormality.

Data collection

The common methods of selecting SNPs are based on haplotype data or genotype data [21–23]. Previous studies have shown that candidate tagging rs12594 and rs16835904 are related to tumor [24]. Four candidate SNPs in the *NR5A2* (rs2246209: chr1:200176405, G > A, 3 Prime UTR Variant; rs1056426: chr1:200177275, $T > C$, 3 Prime UTR Variant) and *RYR2* (rs12594: chr1:237833787, $A > G$, 3 Prime UTR Variant; rs16835904: chr1:237833954, $C > T$, 3 Prime UTR Variant) genes were selected with a minor allele frequency (MAF) great than 0.05 in global population from the 1000 Genome Projects (https://www.internationalgenome.org/).

5 ml of venous blood was collected, DNA was extracted using the whole blood genomic DNA extraction kit (GOLDMAG, Xi´an, China) [25]; primers of 4 sites (rs2246209, rs1056426, rs12594, and rs16835904) were designed by Agena's online software (https://agenacx.com/online-tools/) and genotyping was performed using MassARRAY time flight mass spectrometry array SNP genotyping platform (Agena Bioscience, San Diego, CA, USA) [26], which as we published in the previous article [27].

Statistical analyses

We used SPSS (version 21.0, IBM Corporation, Armonk, NY, USA) and PLINK software (https://zzz.bwh.harvard.edu/plink/ld.shtml) for statistical analysis. Firstly, the

control population was selected to calculate the Hardy Weinberg equilibrium using the Fisher's exact tests to assess whether we were randomly selected control samples [28]. Secondly, logistic regression analysis was used to evaluate the effects of four polymorphic loci on genetic susceptibility to breast cancer, under five genetic models (allele, genotype, recessive, dominant, and additive model). The Odds ratios (ORs) and 95% confidence intervals (CIs) were used to assess the relative risk of breast cancer [29]. At the same time, according to age, tumor size, clinical stage, lymph node metastasis, and the statuses of ER, PR, Cerb-B2, and menopausal for stratified analysis. Bonferroni correction is one of the most important methods to solve the error discovery rate caused by multiple testing. All p values were Bonferroni corrected, and statistical significance was set at $p < 0.0125$ (0.05/4).

Results

Characteristics of cases and controls

The basic clinical information of BC patients is shown in Table 1. 379 patients presented with different distribution, according to age (age < 54, 212 cases; age ≥ 54, 167 cases), menopausal status (premenopausal, 135 cases; postmenopausal, 239 cases), tumor size (size ≤ 2 cm, 164 patients; size > 2 cm, 188 patients), tumor stage (I–II stage, 246 patients; III–IV stage, 116 patients), and status of ER (positive, 274 cases; negative, 105 cases), PR (positive, 240 cases; negative, 139 cases), LNM (positive, 186 cases; negative, 175 cases),and Cerb-B2 (positive, 189 patients; negative, 67 patients).

Basic information and allele frequencies of the *NR5A2* and *RYR2* gene polymorphisms are presented in Table 2. The genotype distribution of all SNPs in control subjects met the HWE ($p > 0.05$). The correlation between *NR5A2* and *RYR2* polymorphisms and BC risk under the allele model is shown in Table 2, unfortunately, there were no differences between SNPs in the *NR5A2,* and *RYR2* genes and BC risk (all $p > 0.05$).

Association between the *NR5A2*, and *RYR2*, genes and the risk of BC

Table 3 shows that rs2246209 in the *NR5A2* gene significantly decreased the risk of BC with the AA genotype (adjusted OR 0.58, 95%CI 0.34–0.99, $p = 0.049$), and in the recessive model (adjusted OR 0.59, 95%CI 0.35–0.99, $p = 0.046$);rs12594 in the *RYR2* gene significantly decreased the risk of BC with the GG genotype (adjusted OR 0.44, 95%CI 0.22–0.88, $p = 0.020$), and in the recessive model (adjusted OR 0.43, 95%CI 0.21–0.85, $p = 0.016$), no significant difference was found for the other SNPs between cases and controls (all $p > 0.05$, Table 3).

Table 1 The comparison of basic characteristics between cases and controls

Characteristics	Case	Control
Number	379	407
Age (mean ± SD)	51.5 ± 10.14	53.04 ± 9.29
< 54	212 (55.9%)	212 (52.1%)
≥ 54	167 (44.1%)	195 (47.9%)
ER status		
Negative	105 (27.7%)	
Positive	274 (72.3%)	
PR status		
Negative	139 (36.7%)	
Positive	240 (63.3%)	
Cerb-B2 status		
Negative	67 (17.7%)	
Positive	189 (49.9%)	
Unavailable	123 (32.4%)	
LNM		
Negative	175 (46.2%)	
Positive	186 (49.1%)	
Unavailable	18 (4.7%)	
Menopausal status		
Premenopausal	135 (35.6%)	
Postmenopausal	239 (63.1%)	
Unavailable	5 (1.3%)	
Tumor size		
≤ 2 cm	164 (43.3%)	
> 2 cm	188 (49.6%)	
Unavailable	27 (7.1%)	
Tumor stage		
I–II	246 (64.9%)	
III–IV	116 (30.6%)	
Unavailable	17 (4.5%)	

ER estrogen receptor, *PR* progesterone receptor, *LNM* lymph nodes metastasis

Stratification analysis of clinical features

We found that rs2246209 in the *NR5A2* gene was related to a lower incidence of BC in people aged ≥ 54 in the homozygote model and recessive model (homozygote model: OR 0.39, 95%CI 0.17–0.89, $p = 0.025$; recessive model: OR 0.39, 95%CI 0.18–0.87, $p = 0.021$); *NR5A2* rs2246209 was associated with a significantly decreased risk of LNM negative BC in the dominant model and log-additive model (dominant model: OR 0.65, 95%CI 0.46–0.94, $p = 0.021$; log-additive model: OR 0.70, 95%CI 0.53–0.93, $p = 0.015$); rs2246209 was correlated with a significant reduction in

Table 2 Basic Information about SNPs in *NR5A2/RYR2* and association with risk of breast cancer in allele model

Gene	SNP ID	Chr	Role	Alleles A/B	MAF		HWE-*p*	OR (95%CI)	*p*	Function
					Cases	Controls				
NR5A2	rs2246209	1q32.1	3'UTR	A/G	0.277	0.312	0.416	0.85 (0.68–1.05)	0.136	DNAse, Motifs changed,
NR5A2	rs1056426	1q32.1	3'UTR	C/T	0.214	0.225	0.776	0.94 (0.74–1.19)	0.589	Motifs changed, GRASP QTL hits,
RYR2	rs12594	1q43	3'UTR	G/A	0.216	0.242	0.176	0.86 (0.68–1.09)	0.227	DNAse, Motifs changed, Selected eQTL hits
RYR2	rs16835904	1q43	3'UTR	T/C	0.214	0.243	0.345	0.85 (0.67–1.07)	0.164	Motifs changed,

SNP single nucleotide polymorphism, *Chr.* Chromosome, *A/B* minor/major, *MAF* minor allele frequency, *HWE* Hardy–Weinberg equilibrium, *OR* odds ratio, *95%CI* 95% confidence interval
$p < 0.05$ indicates statistical significance
Bonferroni's multiple adjustment was applied, with $p < 0.0125$ (0.05/4)

stage I–II BC risk in homozygote model, recessive model, and log-additive model (homozygote model: OR 0.47, 95%CI 0.25–0.90, $p = 0.023$; recessive model: OR 0.52, 95%CI 0.28–0.98, $p = 0.042$; log-additive model OR 0.74, 95%CI 0.57–0.95, $p = 0.018$) (Table 4).

Also, we found that *RYR2* rs12594 was related to BC (Table 5). In homozygote (OR 0.29, 95%CI 0.09–0.91, $p = 0.034$) and recessive model (OR 0.29, 95%CI 0.10–0.90, $p = 0.032$), rs12594 was associated with a reduced risk of BC in women aged ≥ 54; Rs12594 was a protective site for ER-positive (Homozygote: OR 0.35, 95%CI 0.15–0.83, $p = 0.017$; Recessive: OR 0.35, 95%CI 0.15–0.83, $p = 0.014$) and PR-positive (Homozygote: OR 0.23, 95%CI 0.08–0.66, $p = 0.007$; Recessive: OR 0.23, 95%CI 0.08–0.66, $p = 0.006$) BC. Rs12594 in the *RyR2* gene remained significant on the genetic susceptibility of PR-positive BC after Bonferroni correction ($p < 0.0125$). And rs12594 was associated with a significant reduction risk of BC in premenopausal women under the homozygote model (OR 0.37, 95%CI 0.16–0.89, $p = 0.025$) and the recessive model (OR 0.38, 95%CI 0.16–0.88, $p = 0.025$); we also found that rs12594 mutations significantly reduced the incidence of BC with tumor size ≤ 2 cm (Homozygote: OR 0.34, 95%CI 0.11–0.98, $p = 0.045$; Recessive: OR 0.33, 95%CI 0.11–0.95, $p = 0.040$) and tumor stage I–II (Homozygote: OR 0.43, 95%CI 0.19–0.97, $p = 0.043$; Recessive: OR 0.44, 95%CI 0.20–0.98, $p = 0.045$).

Discussion

Through this case–control study, we identified that rs2246209 in *NR5A2* gene, and rs12594 in *RYR2* gene associated with decreased risk of BC.

A number of studies has shown that the occurrence of BC is related to estrogen [30], and the *NR5A2* gene plays an important role in steroid metabolism. Garattini et al. [31] showed that *NR5A2* may be a nuclear receptor with carcinogenic properties. Nadolny and Dong [32] believed that *NR5A2* is a potential BC treatment target. Jiang zhu et al. have shown that by increasing the expression of *NR5A2* and *CREB1*, the low expression of microRNA-2b-3p can enhance the resistance of tamoxifen in breast cancer [33]. Studies have shown that nuclear receptor *NR5A2* is involved in the main prognosis of invasive ductal breast cancer [14]. These evidences indicated that there is a relationship between *NR5A2* and BC. However, it is still unclear whether the polymorphism of *NR5A2* is related to the risk of BC. In our study, we found that mutations of the rs2246209 locus in the *NR5A2* gene reduced the risk of BC and clarified the association between *NR5A2* gene and BC susceptibility in Chinese Han female. Pang et al. [34] determined that the expression pattern of the *NR5A2* gene in BC affects the invasiveness of

Table 3 Relationship between *NR5A2/RYR2* gene polymorphisms and risk of breast cancer under multiple models of inheritance

SNP ID	Model	Genotype	Control	Case	Adjusted OR (95%CI)	*p*
NR5A2 rs2246209	Codominant	G/G	192	193	1.00	**0.049**
		G/A	164	162	0.98 (0.73–1.32)	
		A/A	42	24	**0.58 (0.34–0.99)**	
	Dominant	G/G	192	193	1.00	0.456
		G/AA/A	206	186	0.90 (0.68–1.19)	
	Recessive	G/GG/A	356	355	1.00	**0.046**
		A/A	42	24	**0.59 (0.35–0.99)**	
	Log-additive	–	–	–	0.85 (0.68–1.06)	0.146
RYR2 rs12594	Codominant	A/A	239	227	1.00	**0.020**
		A/G	139	140	1.06 (0.78–1.42)	
		G/G	29	12	**0.44 (0.22–0.88)**	
	Dominant	A/A	239	227	1.00	0.714
		A/GG/G	169	152	0.95 (0.71–1.27)	
	Recessive	A/AA/G	378	367	1.00	**0.016**
		G/G	29	12	**0.43 (0.21–0.85)**	
	Log-additive	–	–	–	0.86 (0.68–1.09)	0.219

OR odds ratio, *CI* confidence interval

$p < 0.05$ indicates statistical significance

Bold values indicate a significant difference

Bonferroni's multiple adjustment was applied, with $p < 0.0125$ (0.05/4)

Table 4 Relationship between *NR5A2* rs2246209 and risk of breast cancer under multiple models of inheritance

Variables	Heterozygote		Homozygote		Dominant		Recessive		Log-additive	
	OR (95%CI)	*p*	OR (95%CI)	*p*	OR (95%CI)	*p*	OR (95%CI)	*p*	OR (95%CI)	*p*
Age										
< 54	0.98 (0.65–1.46)	0.918	0.88 (0.41–1.85)	0.729	0.96 (0.65–1.42)	0.847	0.89 (0.43–1.82)	0.740	0.95 (0.70–1.30)	0.769
≥ 54	1.00 (0.64–1.56)	0.991	**0.39 (0.17–0.89)**	**0.025**	0.85 (0.56–1.29)	0.449	**0.39 (0.18–0.87)**	**0.021**	0.77 (0.55–1.06)	0.104
LNM										
Positive	1.37 (0.95–1.97)	0.091	0.71 (0.35–1.42)	0.331	1.24 (0.87–1.76)	0.234	0.61 (0.31–1.18)	0.141	1.04 (0.79–1.36)	0.795
Negative	0.69 (0.47–1.01)	0.058	0.50 (0.25–1.01)	0.054	**0.65 (0.46–0.94)**	**0.021**	0.58 (0.29–1.16)	0.124	**0.70 (0.53–0.93)**	**0.015**
Tumor stage										
I–II	0.80 (0.57–1.12)	0.188	**0.47 (0.25–0.90)**	**0.023**	0.73 (0.53–1.01)	0.057	**0.52 (0.28–0.98)**	**0.042**	**0.74 (0.57–0.95)**	**0.018**
III–IV	1.51 (0.97–2.34)	0.066	1.04 (0.48–2.23)	0.929	1.42 (0.93–2.16)	0.107	0.84 (0.41–1.74)	0.635	1.18 (0.86–1.61)	0.311

LNM lymph node metastasis, *OR* odds ratio, *CI* confidence interval

$p < 0.05$ indicates statistical significance

Bold values indicate a significant difference

Bonferroni's multiple adjustment was applied, with $p < 0.0125$ (0.05/4)

BC cells. Bianco et al. [35] believed that the *NR5A2* gene is involved in the regulation of transcriptional processes in BC cells. We know that non-coding RNA can bind specifically to the 3′UTR region of the target gene. The single nucleotide polymorphism in the target gene 3′UTR region can affect the binding of the target gene to the gene, leading to changes in the expression of the target gene, thus affecting the occurrence of disease. Studies found that the *NR5A2* gene was highly expressed in breast cancer tissues, and we obtained the same results by bioinformatics analysis (GEPIA: https://gepia.cancer-pku.cn/detail.php?gene=#boxplot), while the rs2246209 locus is located in the *NR5A2* 3′UTR region, therefore, we hypothesized that rs2246209 may affect the expression level of *NR5A2* in breast cancer and inhibit the occurrence of breast cancer.

Some studies have shown that *RyR* and *RYR3* genes are related to the prognosis, risk and calcification of breast cancer, respectively [19, 20]. Kobylewski et al. [36] found that the invasiveness of female BC was positively correlated with the expression of *RYR2* in BC tissues. In the process of

Table 5 Relationship between rs12594 in *RYR2* and risk of breast cancer under multiple models of inheritance

Variables	Heterozygote		Homozygote		Dominant		Recessive		Log-additive	
	OR (95%CI)	*p*	OR (95%CI)	*p*	OR (95%CI)	*p*	OR (95%CI)	*p*	OR (95%CI)	*p*
Age										
< 54	1.16 (0.77–1.74)	0.484	0.60 (0.24–1.49)	0.272	1.07 (0.72–1.58)	0.745	0.57 (0.23–1.39)	0.216	0.97 (0.70–1.34)	0.844
≥ 54	0.98 (0.63–1.53)	0.944	**0.29 (0.09–0.91)**	**0.034**	0.85 (0.56–1.31)	0.471	**0.29 (0.10–0.90)**	**0.032**	0.77 (0.54–1.10)	0.151
ER status										
Positive	1.04 (0.75–1.44)	0.797	**0.35 (0.15–0.83)**	**0.017**	0.93 (0.68–1.27)	0.629	**0.35 (0.15–0.81)**	**0.014**	0.83 (0.64–1.08)	0.174
Negative	1.10 (0.70–1.73)	0.677	0.69 (0.25–1.85)	0.455	1.03 (0.67–1.59)	0.894	0.66 (0.25–1.75)	0.405	0.96 (0.68–1.36)	0.818
PR status										
Positive	1.00 (0.72–1.41)	0.982	**0.23 (0.08–0.66)**	**0.007**	0.87 (0.63–1.21)	0.415	**0.23 (0.08–0.66)**	**0.006**	0.78 (0.59–1.03)	0.076
Negative	1.17 (0.78–1.75)	0.458	0.85 (0.37–1.93)	0.694	1.11 (0.75–1.64)	0.594	0.80 (0.36–1.79)	0.586	1.03 (0.76–1.41)	0.839
Menopausal status										
Postmenopausal	0.98 (0.69–1.39)	0.910	**0.37 (0.16–0.89)**	**0.025**	0.87 (0.63–1.22)	0.424	**0.38 (0.16–0.88)**	**0.025**	0.80 (0.61–1.06)	0.124
Premenopausal	1.25 (0.78–1.99)	0.356	0.62 (0.21–1.86)	0.395	1.15 (0.73–1.80)	0.558	0.57 (0.19–1.68)	0.310	1.02 (0.70–1.47)	0.931
Tumor size										
≤ 2 cm	1.06 (0.72–1.55)	0.764	**0.34 (0.11–0.98)**	**0.045**	0.94 (0.65–1.35)	0.724	**0.33 (0.11–0.95)**	**0.040**	0.84 (0.62–1.14)	0.263
> 2 cm	1.04 (0.72–1.50)	0.840	0.60 (0.27–1.36)	0.225	0.96 (0.68–1.37)	0.842	0.59 (0.27–1.33)	0.205	0.91 (0.68–1.21)	0.507
Tumor stage										
I–II	0.95 (0.68–1.34)	0.783	**0.43 (0.19–0.97)**	**0.043**	0.86 (0.62–1.20)	0.378	**0.44 (0.20–0.98)**	**0.045**	0.81 (0.62–1.06)	0.132
III–IV	1.25 (0.81–1.92)	0.316	0.53 (0.18–1.55)	0.245	1.13 (0.74–1.71)	0.580	0.48 (0.17–1.40)	0.181	0.98 (0.70–1.38)	0.927

OR odds ratio, *CI* confidence interval, *ER* estrogen receptor, *PR* progesterone receptor

$p < 0.05$ indicates statistical significance

Bold values indicate a significant difference

Bonferroni's multiple adjustment was applied, with $p < 0.0125$ (0.05/4)

epithelial–mesenchymal transition in BC, the most significant change in gene expression is *RYR2* [37], indicating that the expression of *RYR2* gene is involved in the metastasis of BC. The rs12594 locus is located in the *RYR2* 3′UTR region, and previous studies have shown that candidate tagging rs12594 and rs16835904 are related to tumor. However, the mechanism of *RyR2* polymorphism in breast cancer is still unclear. In our study, we also found that rs12594 on *RYR2* gene was significantly associated with BC risk, and it was the first time clearly indicating the relationship between *RYR2* gene and BC risk. Therefore, we hypothesized that 12,594 may affect the expression level of *RYR2* in breast cancer and inhibit the occurrence of breast cancer.

However, it is not clear whether the mutation of *NR5A2* and *RYR2* genes really affects expression. After that, it is necessary to analyze the expression of *NR5A2* and *RYR2* genes in cancer and normal tissues by real-time fluorescence quantitative PCR, and further analyze the influence of different genes on the expression of breast cancer, and further clarify the role of two these genes in the development of breast cancer. Furthermore, our research suggested that age, tumor status, tumor stage, and the statuses of ER, PR, LNM, Cerb-B2, and menopausal play a key role in the susceptibility of BC. After Bonferroni's correction, *RyR2* gene rs12594 still had a significant effect on the genetic susceptibility of PR-positive BC ($p < 0.0125$), while the other loci in our study were not found to be related to the risk of BC. This may be due to our strict SNP screening criteria and small samples. In addition, the Bonferroni correction adjusts the value of alpha according to the number of experiments carried out, so it is conservative; in some cases, due to type II errors, the truly significant differences may be considered insignificant [38]. Huang et al. [39] demonstrated that the risk of BC is affected by clinical features such as ER, PR, LNM, and tumor stage. It is speculated that the cause may be different hormone levels in patients with different clinical phenotypes, and estrogen is essential for BC development [40]. Wang et al. [41] believed that estrogen is a dangerous biomarker for BC development. Suba [42] revealed that estrogen tolerance in BC patients significantly affects treatment outcomes.

This study provides an evidence for *NR5A2* rs2246209 and *RYR2* rs12594 decreased the risk of breast cancer. However, further studies are warranted on larger patients from other ethnic groups to confirm our results. And, through in vivo and in vitro experiments, explore the molecular mechanism of *NR5A2* rs2246209 and *RYR2* rs12594 to reduce the risk of breast cancer.

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Compliance with ethical standards

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Our present study was approved by the Ethics Committee of Shaanxi Provincial Cancer Hospital. Informed consent forms were signed by all participants.

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